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Title: A HUMAN GENE RELATED TO BUT DISTINCT FROM EGF RECEPTOR GENE

Background of the Invention

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Technical Field

The present invention is related to the cloning, isolation and partial characterization of a hitherto unidentified human gene. More particularly, the present invention is related to the preparation and identification of a v-erbB related human gene that is a new member of the tyrosine kinase encoding family of genes and is amplified in a human mammary carcinoma.

State of the Art

A number of genes have been identified as retroviral oncogenes that are responsible for inducing tumors in vivo and transforming cells in vitro (Land et al., Science 222:771-778, 1983). Some of them apparently encode transforming proteins that share a kinase domain

homologous to that of pp60^{src}, a tyrosine-specific protein kinase. The cellular cognate, encoded by the c-src gene, also exhibits tyrosine-specific kinase activity. Of particular interest is the fact that tyrosine-specific kinases are also encoded by other genes for several receptors for polypeptide growth factors, including the receptors for epidermal growth factor (EGF) (Cohen et al., J. Biol. Chem. 255:4834-4842, 1980), platelet-derived growth factor (PDGF) (Nishimura et al., Proc. Natl. Acad. Sci. USA 79:4303-4307, 1982), insulin (Kasuga et al., Nature 298:667-669, 1982), and insulin-like growth factor I (Rubin et al., Nature 305:438-440, 1983). This implies a possible link between the action of the growth factor-receptor complex and the oncogene products with tyrosine-specific kinase activity.

Recent analysis of the v-erbB gene and the EGF receptor gene indicates that the v-erbB gene is a part of the EGF receptor gene and codes for the internal domain and transmembrane portion of the receptor (Yamamoto et al., Cell 35:71-78, 1983; Downward et al., Nature 307:521-527, 1984; Ullrich et al., Nature 309:418-425, 1984). These findings, together with the extensive identity of the amino acid sequences of the v-sis protein and platelet-derived growth factor (Waterfield et al.,

Nature 304:35-39, 1983; Doolittle et al., Science 221:275-277, 1983), suggest that some viral oncogene products mimic the action of the polypeptide growth factor-receptor complex in activating a cellular pathway involved in cell proliferation and tumor formation.

Genetic alterations affecting proto-oncogenes of the tyrosine kinase family may play a role in spontaneous tumor development. A specific translocation affecting the c-abl locus, for example, is associated with chronic myelogenous leukemia (de Klein et al., Nature 300:765, 1982; Collins et al., Proc. Natl. Acad. Sci. USA 80:4813, 1983). Several recent studies have also documented amplification or rearrangement of the gene for the EGF receptor in certain human tumors (Libermann et al., Nature 313:144, 1985), or tumor cell lines (Ullrich et al., Nature 309:418, 1984; Lin et al., Science 224:843, 1984). However, a gene that is a new member of the tyrosine kinase family and is amplified in a human mammary carcinoma and is closely related to, but distinct from the EGF receptor gene, has not heretofore been known.

SUMMARY OF THE INVENTION

It is, therefore, an object of the present invention to provide a novel, cloned, human gene having the nucleotide sequence as shown in Fig. 1 and described more fully herein infra.

It is a further object of the present invention to provide products, e.g. various RNAs and/or polypeptides encoded by the cloned gene.

It is a still further object of the present invention to provide antibodies, either polyclonal or monoclonal, directed against the protein product encoded by said gene and a diagnostic kit containing said antibodies for the detection of carcinomas.

It is another object of the present invention to provide complementary DNA (cDNA) clones homologous to the messenger RNA (mRNA) encoded by the cloned gene, said cDNA clones being capable of expressing large amounts of corresponding protein in a heterologous vector system, such as bacteria, yeast, and the like.

It is yet another object of the present invention to produce a transformed cell or organism capable of

expressing said gene by incorporating said gene or a part thereof into the genome of said cell, vector or organism.

It is a still further object of the present invention to provide nucleic acid probes and/or antibody reagent kits capable of detecting said gene or a product thereof.

Other objects and advantages of the present invention will become apparent as the detailed description of the invention proceeds.

BRIEF DESCRIPTION OF DRAWINGS

These and other objects, features and many of the attendant advantages of the invention will be better understood upon a reading of the following detailed description when considered in connection with the accompanying drawings wherein:

~~Fig. 1 shows a characteristic fragment produced by EcoRI restriction of the cloned gene of the present invention; detection of v-erbB- and pMAC 117-specific gene fragments in normal human placenta, A431 cells, or human mammary carcinoma MAC117. DNA (15 µg) was cleaved with Eco RI, separated by electrophoresis in agarose~~

gels, and transferred to nitrocellulose paper (Southern J. Mol. Biol. 98:503, 1975). Hybridization to the ^{32}P-labeled probe (Rigby et al., J. Mol. Biol. 113:237, 1977) was conducted in a solution of 40 percent formamide, 0.75M NaCl, 0.075M sodium citrate, at 42°C (Wahl et al., Proc. Natl. Acad. Sci. USA 76:3683, 1979). The v-erbB probe (A) was a mixture of the 0.5-kbp Bam HI-Bam HI fragment and 0.5-kbp Bam HI-Eco RI fragment of avian erythroblastosis proviral DNA. The pMAC117 probe (B) was a 1-kbp Bgl I-Bam HI fragment. After hybridization, the blots were washed first in 0.3M NaCl plus 0.03M sodium citrate at room temperature, and then in 0.015M NaCl, 0.0015M sodium citrate, and 0.1 percent sodium dodecyl sulfate at 42°C (A) or at 52°C (B). Hybridization was detected by autoradiography.

Fig. 2 shows the gel electrophoretic properties of specific gene fragments; Restriction-site map of MAC117 and plasmid pMAC117. A, Acc 1; B, Bam HI; Bg, Bgl I; N, Nco I; R, Eco RI; X, Xba I; Xh, Xho I. The sites were located by electrophoretic analysis of the products of single and double digestion. Regions homologous to v-erbB or human repetitive sequences (region flanked by arrows) were located by Southern blot hybridization

(Southern, J. Mol. Biol. 98:503, 1975)with the v-erb B probe or total human DNA made radioactive by nick translation (Rigby et al., J. Mol. Biol. 113:237, 1977). Hybridization conditions were as described in Fig. 1A. The nucleotide sequence of pMAC117 between the Acc I site and the Nco I sites and regions of encoded amino acid sequence homologous to the EGF receptor are shown. The AG or GT dinucleotides flanking the putative coding regions are underlined. To determine the sequence, Nco I, Hinf I, and Sau 96 I fragments were labeled at the 3' termini by means of the large fragment of E. coli DNA polymerase, separated into single strands by gel electrophoresis, and chemically degraded (Maxam et al., Proc. Natl. Acad. Sci. USA 74:560, 1977).

Fig. 3 shows a comparison of the putative encoded amino acid sequence of various polypeptide products, and comparison of the putative encoded amino acid sequence in pMAC117 with known tyrosine kinase sequences. Black regions represent homologous amino acids. Differing amino acid residues are shown in one-letter code (A, alanine; C. cysteine, D. aspartic acid; E. glutamic acid; F. phenylalanine; G. glycine; H. histidine; I. isoleucine; K. lysine; L. leucine; M. methionine;

N. asparagine; P. proline; Q. glutamine; R. arginine; S. serine; T. threonine; V. valine; W. tryptophan; Y. tyrosine). Amino acid positions conserved in all sequences are denoted by *. The tyrosine homologous to that autophosphorylated by the v-src protein (Smart et al., Proc. Natl. Acad. Sci. USA 78:6013, 1981) is shown by an arrow. The v-abl sequence contains a tyrosine residue in this region displaced by two positions. The amino acid sequences of human EGF receptor, v-src, v-abl, v-fms, and human insulin receptor were aligned by the computer program described by Ullrich et al., Nature 313:756, 1985 which is incorporated herein by reference. The homology observed with the predicted amino acid sequences of v-yes and v-fes was 51 percent and 48 percent, respectively.

Fig. 4 shows the distinction between λMAC117 and human EGF receptor genes by the detection of distinct messenger RNA species derived from the λMAC117 gene and the human EGF receptor gene. Polyadenylated messenger RNA of A431 cells was separated by denaturing gel electrophoresis in formaldehyde (Lehrach et al., Biochemistry 16:4743, 1977), transferred to nitrocellulose (Southern, J. Mol. Biol. 98:503, 1975), and hybridized under stringent conditions (50 percent formamide, 0.75 M NaCl, 0.075M sodium citrate, at 42°C)

with ^{32}P-labeled probe from pMAC117 (Bgl I-Bam HI fragment) or human EGF receptor complementary DNA (pE7s 2-kb Cla I inserted fragment). Filters were washed under conditions of high stringency (0.015M NaCl plus 0.0015M sodium citrate at 55°C). Hybridization was detected by autoradiography with exposure times of 4 hours for the pMAC117 probe and 1 hour for the human EGF receptor probe.

Fig. 5A shows the restriction map of complementary DNA of MAC117 encompassing the entire coding region of the gene. Clone pMAC137 was isolated from an oligo dT primed normal human fibroblast cDNA library (Okyama et al., Mol. Cell. Biol. 3, 280, 1983) using a 0.8-kbp Acc I fragment from the 3' terminus of pMAC117 as probe. Clones λMAC30, λMAC10', and λMAC14-1 were subsequently isolated from a randomly primed MCF-7 cDNA library (Walter et al., Proc. Natl. Acad. Sci. USA, 82, 7889, 1985) using cDNA fragments as probes. Restriction sites: B - Bam HI, BII - Bst EII, E - Eco RI, N - NCO I, P - Pst I, Sm - Sma I, Sp - Sph I, and St - Stu I.

Fig. 6 shows the overexpression of MAC117 in RNA in human mammary tumor cell lines. (A) Northern blot analysis. Total cellular RNA (10 µg) of mammary tumor cell lines, normal fibroblasts M413 and HBL100 was hybridized with a cDNA probe derived from the 5' end of the coding region (Fig. 5B, probe a). M413 and HBL100 cells contain specific mRNA detectable after longer autoradiographic exposures. Similar results were obtained when probe b or c (Fig. 5B) was employed for hybridization. (B) Quantitation of mRNA levels. Serial 2-fold dilutions of total RNA were applied to nitrocellulose. Replicate filters were hybridized with either a cDNA probe (Fig. 5B, probe b) or human β-actin which served as control for RNA amounts present on the nitrocellulose filter. Relative amounts detected with each probe are indicated in comparison to the hybridization signals observed in normal human fibroblast M413.

Fig. 7 shows the 185-kDal protein specific for MAC117 and its overexpression in human mammary tumor cell lines. 40 µg total cellular protein was separated by electrophoresis and transferred to nitrocellulose filters. The protein was detected with an antipeptide antibody coupled to ^{125}I protein A. The

specificity of antibody detection was determined by pre-incubation of the antibody with excess amounts of peptide prior to immunodetection. (+) preincubation with peptide, (-) no peptide. In panel B, nonspecific bands at 100 kd are observed in longer exposures of peptide blocked immunoblots (panel A).

Fig. 8 shows the gene amplification of MAC117 in 4 mammary tumor cell lines and the absence of MAC117 gene amplification in 4 other mammary tumor cell lines overexpressing MAC117 mRNA. (A) Southern blot analysis. For each line 10 µg genomic DNA were restricted with Xba I and hybridized with a probe comprising the entire coding region of MAC117 (Fig. 5B, probe c). Hind III restriction fragments of lambda DNA served as mol. wt. standards. (B) DNA dot-blot analysis. Genomic DNA (10 µg) digested with Eco RI was applied in serial 2-fold dilutions to nitrocellulose filters. Filters were hybridized either with a probe specific for MAC117 (Fig. 5B, probe b) or mos, which served as a control for DNA amounts applied to replicate nitrocellulose filters. Gene copy numbers of MAC117 relative to M413 indicate the minimal extent of gene amplification detected in DNA from mammary tumor cell lines.

Fig. 9 depicts the construction of expression vectors for the human MAC117 cDNA. A Nco I-Mst II fragment encompassing the entire open reading frame was cloned under the transcriptional control of either the SV40 early promoter or MuLV LTR. Symbols:  , erbA-erbB intergenic region of pAEV11 containing the 3' splice acceptor site; N = Nco I, Sp = Sph I, M = Mst II, St = Stu I, H = Hind III, Sm = Sma I, P = Pst I, B = BamH I, X = Xho I. Sites indicated in parenthesis were not reconstituted after the cloning procedures.

Table 1 compares transformation characteristics of NIH/3T3 cells transfected with vectors generating different expression levels of the MAC117 coding sequence.

Table 1.

DNA transfectant[a]	Specific transforming activity[b] (ffu/pM)	Colony-forming efficiency in agar (%)[c]	Cell number required for 50% tumor incidence[d]
LTR-1/MAC117	4.1×10^4	45	10^3
SV40/MAC117	$<10^0$	<0.01	$>10^6$
LTR/erbB	5.0×10^2	20	5×10^4
LTR/ras	3.6×10^4	35	10^3
pSV2/gpt	$<10^0$	<0.01	$>10^6$

[a]All transfectants were isolated from plates which received 1 µg cloned DNA and were selected by their ability to grow in the presence of killer HAT medium (Mulligan et al., Proc. Natl. Acad. Sci. USA 78, 2072, 1981).

[b]Focus-forming units were adjusted to ffu/pM of cloned DNA added based on the relative molecular weights of the respective plasmids.

[c]Cells were plated at 10-fold serial dilutions in 0.33% soft agar medium containing 10% calf serum. Visible colonies comprising >100 cells were scored at 14 days.

[d]NFR nude mice were inoculated subcutaneously with each cell line. Ten mice were tested at cell concentrations ranging from 10^6 to 10^3 ~~to 10^{-3}~~ cells/mouse. Tumor formation was monitored at least twice weekly for up to 30 days.

Fig. 10 shows the comparison of the levels of MAC117 proteins in LTR-1/erbB-2 transformed NIH/3T3 cells and human mammary tumor lines by immunoblot analysis. Varying amounts of total cellular protein were separated by electrophoresis and transferred to nitrocellulose filters. The MAC117 protein was detected with rabbit anti-peptide serum coupled to ^{125}I protein A as previously described.

DETAILED DESCRIPTION OF INVENTION

The above and other objects and advantages of the present invention are achieved by a cloned human gene having the nucleotide sequence as shown in Fig. 1. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned under the "Brief Description of Drawings" and hereunder are incorporated herein by reference. Unless defined otherwise, all technical or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

Cells and Tissues:

Preparation of High Molecular Weight DNA

1. From A431 cells:

A431 carcinoma cells were established in culture and maintained in Dulbecco's modified Eagle's medium with 10% fetal calf serum.

Cells were grown to 90% confluence in four 175 cm^2 tissue culture flasks, washed twice with phosphate buffered saline (Gibco Biochemicals), then lysed in 10 mM Tris (pH 7.5), 150 mM NaCl, 50 mM ethylenediamine-tetraacetate (EDTA) and 0.5% sodium dodecyl sulfate (SDS). Proteinase K (Boehinger Mannheim) was added to a concentration of 0.1 mg/ml and the cell extracts digested for 3 hours at 50°C. DNA was extracted 3 times with phenol and once with $CHCl_3$. DNA was precipitated with 2 volumes of ethanol, spooled and resuspended in 20 ml of 10 mM Tris-HCl (pH 7.5), 1 mM EDTA. The solution was then made 10 µg/ml with (DNase free) RNase (Boehinger Mannheim) and incubated for 2 hr at 50°C. NaCl was added to 0.5 M and the solution extracted with phenol followed by $CHCl_3$. DNA was precipitated with 2 volumes of ethanol, spooled, and resuspended in 10 mM Tris, 1 mM EDTA. The concentration was determined by routine spectrophotometric procedure at 260 nm wavelength.

2. From tissues:

Two grams original mass of primary tumor (designated MAC117 obtained from Memorial Sloan-Kettering Cancer Center Specimen code 31-26606) ~~was~~ were pulverized in a mortar and pestle at liquid nitrogen temperature, suspended in 10 ml of 10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 2 mM EDTA, reacted with proteinase K at 500 µg/ml (Boehinger Mannheim) and SDS at 0.5% at 37°C for 10 hr. The solution was then extracted twice with phenol and twice with the mixture of phenol:$CHCl_3$: isoamyl alcohol at 25:24:1 and once with $CHCl_3$:isoamyl alcohol (24:1). DNA was precipitated by 2 volumes of ethanol removed by spooling, and resuspended in 1 mM Tris-HCl (pH 7.5), 0.2 mM EDTA.

Electrophoretic analysis of DNA fragments using "Southern hybridization"

1. Restriction enzyme cleavage

Each sample of DNA (15 µg) was digested in 0.4 ml of 100 mM Tris-HCl (pH 7.5), 50 mM NaCl, 5 mM $MgCl_2$, 100 ug/ml bovine serum albumin and 30 units of restriction enzyme (New England Biolabs) for 2 hr at 37°C. Following reaction, 10 µg of tRNA was added and the solution extracted once with an equal volume of a mixture of phenol and $CHCl_3$ (1:1). Nucleic acids were

precipitated from the aqueous phase by addition of 2 volumes of ethanol. Following centrifugation for 10 min at 12,000 x g (Eppendorf microfuge) the samples were washed once with 80% ethanol, dried to remove ethanol, and resuspended in 40 µl distilled H_2O.

2. Agarose gel electrophoresis

DNA samples were made 40 mM Tris acetate (pH 7.2), 20 mM Na acetate, 1 mM EDTA, 5.0% glycerol, 0.05% bromophenol blue. Electrophoresis was conducted in a BRL H4 apparatus containing 400 ml 0.8% agarose, 40 mM Tris acetate (pH 7.2), 20 mM Na acetate, 1 mM EDTA and 1 µg/ml ethidium bromide for about 16 hr at about 50 volts following conventional procedure. DNA was detected by irradiation with ultraviolet light.

3. Transfer to nitrocellulose

The agarose gel was treated twice for 15 min in 1 liter of 0.5 M NaOH, 1.5 M NaCl, then twice for 30 min with 1 M NH_4Ac, 20 mM NaOH. The agarose gel was then placed on a stack of filter paper saturated with 1 liter of 1 M NH_4Ac, 20 mM NaOH. A sheet of nitrocellulose membrane (0.45 µm pore size Schleicher & Schuell) was placed on top of the gel followed by dry filter paper. Transfer was allowed to occur overnight. DNA was fixed to nitrocellulose by baking at 80°C in vacuo for 2 hr.

Hybridization to RNA and DNA blots

Hybridization was conducted in 20 ml of 40% formamide, 0.75 M NaCl, 0.075 M Na citrate, 0.05% BSA, 0.05% polyvinyl pyrolidone, 0.05% Ficol 400 and 20 μg/ml sheared denatured calf thymus DNA. All hybridization was conducted for 16 hr at 42°C in a water bath. Following hybridization, nitrocellulose membranes were washed 2 times for 20 min in 1 liter of 0.3M NaCl, 30 mM Na citrate, followed by washes in 15 mM NaCl, 1.5 mM Na citrate, first with and then without 0.1% sodium dodecyl sulfate. These final washes were at 42°C for v-erbB probes and at 52°C with pMAC117 and pE7 probes, vide infra. Autoradiography was conducted at -70°C with Kodak XAR5 film. Exposure times were 2 hr for Fig. 2A and 20 min for Fig. 2B, 40 min for EGF receptor probe on Fig. 4, and 4 hr for the pMAC117 probe of Fig. 4.

Generation of

A nucleic acid probe is defined as a fragment of DNA or RNA whose nucleotide sequence has at least partial identity with the sequence of the gene or its messenger RNA so as to enable detection or identification of the gene. Since a gene may have several fragments, there could be a plurality of probes for detecting the gene.

The probes used were the 0.5-kb Bam HI to Bam HI fragment combined with the 0.5-kb Bam HI to Eco RI

fragment of the v-erbB gene of AEV 11; the 1-kb BglI to Bam HI fragment of pMAC117; and the 2-kb Cla I fragment of pE7 as described by Xu, et al., (Nature 309:806, 1984). DNA fragments were isolated by gel electrophoresis in 1% low melting point agarose gels (Bethesda Research Labs) in 40 mM Tris acetate, 20 mM Na acetate, 1 mM EDTA, followed by melting of the gel at 70°C and extraction with phenol followed by $CHCl_3$ and ethanol precipitation. DNAs were made radioactive by using a nick-translation kit (Amersham) in which 50 µl reactions contained 250 µCi αP^{32}dCTP (Amersham) and 0.5 µg DNA. Radioactive probe DNA was purified from unincorporated nucleotides by 2 cycles of ethanol precipitation. Yields were above 2 x 10^8 cpm/µg DNA. Before hybridization all probes were made single-stranded by treatment with 90% formamide.

RNA electrophoresis and transfer to nitrocellulose

RNA samples (5 µg A431 polyadenylated RNA, obtained from National Institutes of Health, Bethesda, MD 21218) were treated for 5 min at 50°C in 50% formamide, 6.7% formaldehyde, 20 mM Mops (pH 7.0) (Sigma Biochemicals), 5 mM Na acetate, 1 mM EDTA in 25 µl total volume. Electrophoresis was conducted in BRL H4 apparatus in 250ml of 1.5% agarose, 20 mM Mops (pH 7.0), 5 mM Na

acetate, 1 mM EDTA, 1 μg/ml ethidium bromide at 40 volts for 16 hr. RNA was detected using ultraviolet light. The gel was soaked for 30 min at 20°C in 50 mM NaOH, followed by two 30 min washes in 1 M Tris (pH 7.5), followed by 30 min in 3 M NaCl, 0.3 M Na citrate. Transfer to nitro-cellulose was accomplished by placing the gel atop a stack of filter paper saturated with 1.5 M NaCl, 0.15 M Na citrate, followed by 0.45 μM pore size nitrocellulose (Schleicher and Schuell), followed by dry filter paper. Transfer was allowed to proceed for 16 hr. The nitrocellulose filter was washed twice for 20 min in 0.3 M NaCl, 30 mM Na citrate. RNA was fixed to the paper by baking at 80°C in vacuo for 2 hr.

DNA sequence analysis

DNA fragments containing the AccI-NcoI region (Fig. 1) were digested with either Nco I, Hinf I or Sau 96I (New England Biolabs). These fragments were end-labeled in reactions of 50 μl containing 50 mM Tris-HCl (pH 7.2), 10 mM $MgCl_2$, 0.1 mM dithiothreitol, 50 μg/ml BSA, 10 μCi α-^{32}P-dXTP (Amersham--where x represents the correct nucleotide for fill-in), 2 units E. coli DNA polymerase large fragment (New England Biolabs). Following labeling, single-stranded material was prepared by electrophoresis. Samples were denatured in 30%

dimethyl sulfoxide, 1 mM EDTA and 0.05% bromophenol blue at 90°C for 2 hr. Samples were chilled and electrophoresed in acrylamide gels in a Bethesda Research Labs apparatus. DNA was detected by autoradiography and isolated by elution into 10 mM Tris-HCl (pH 7.0), 1 mM EDTA. Chemical degradation of DNA for sequence analysis was conducted using standard procedures. Cleavage at guanine (G) residues was conducted by reaction with dimethyl sulfonate at 22°C for 10 min. Cleavage at adenine (A) residues was conducted by 12 min reaction at 90°C in 1.5 M NaOH, 1 mM EDTA. Cleavage at cytosine (C) residues was conducted using hydrazine in 2 M NaCl for 13 min at 22°C. Cleavage at thymine (T) residues was conducted using hydrazine with no added NaCl for 10 min at 22°C. Following cleavage, all reactions were twice precipitated using ethanol and thoroughly dried. All samples were reacted with 1 M piperidine at 90°C for 30 min. Piperidine was removed by evaporation in a Savant speed vac concentrator. Fragments were separated by electrophoresis in acrylamide gels (BRL HO apparatus) in 8 M urea, 50 mM Tris-borate (pH 8.3), 1 mM EDTA. Detection of degraded ladder was by autoradiography using Kodak XAR5 film at -70°C.

Cloning of λMAC117

High molecular weight DNA (6 μg) from tumor MAC117 (see above) was digested with 12 units restriction enzyme Eco RI (New England Biolabs) in a volume of 100 ul for about one hour at 37°C. DNA was obtained by phenol $CHCl_3$ extraction and ethanol precipitation and resuspended in water at a concentration of 0.1 μg/ml. This DNA (0.2 μg) was ligated to λwes λB arms (Bethesda Research Labs) (1 μg) using T4 DNA ligase (New England Biolabs) in a total volume of 20 ml [50 mM Tris-HCl pH 7.4, 10 mM $MgCl_2$ 10 mM dithiothretol, 0.5 mM spermidine, 1 mM ATP]. This mixture of ligated DNAs was packaged into infectious bacteriophage particles using the Packagene system (Promega Biotec). These particles were used to infect bacteria BNN45 and about 8×10^5 individual phage plaques were obtained.

These phage plaques were screened for individual plaques containing DNA homologous to the v-erbB probes (described above) using standard procedures. Briefly, bacterial culture plaques containing approximately 15,000 plaques were grown overnight. Sterile nitrocellulose discs (Scheicher and Schuell) were applied to the dish, removed and allowed to air dry for about 90 minutes. The

discs were then treated with 0.2 M NaOH, 1.5 M NaCl followed by 0.4 M Tris-HCl pH 7.5 followed by 0.3 M NaCl 0.03 M Na citrate and baked in vacuo for two hours at 80°C. These discs were then exposed to hybridization and washing conditions identical to those described for Fig. 1 using the identical v-erbB probe. Washing conditions were also identical to those for Fig. 1. Hybridization was detected by autoradiography at -70°C for 16 hours. Single hybridizing phage plaques were obtained by three successive hybridization experiments (as described above) to isolate a pure phage culture.

~~To clone the 6-kbp fragment,~~ DNA from MAC117 was digested with Eco RI, then ligated into bacteriophage λgtWES, packaged in vitro, and transferred to Escherichia coli (E. coli) strain BNN45 by infection following standard techniques well known in the art. A library of 4×10^5 bacteriophages was screened by plaque hybridization with radioactive v-erbB DNA. Ten of 14 hybridizing phages contained a 6-kbp Eco RI fragment. Figure 2 shows the physical map of one of these phages, λMAC117, and pMAC117, a PUC12 subclone containing a 2-kbp Bam HI fragment of λMAC117 that hybridized with v-erbB probes. The region of pMAC 117 to which v-erbB hybridized most intensely was flanked by Acc I and Nco I sites. Human repetitive sequences were also localized (Fig. 2, region demarcated by arrows).

A deposit of pMAC117 cloned in E. coli has been made at the American Type Culture Collection (ATCC), Bethesda, Md. under accession number 53408. Upon issuance of a patent, the culture will continue to be maintained for at least 30 years and made available to the public without restriction subject, of course, to the provisions of the law in this respect.

As shown in Fig. 1A, DNA prepared from tissue of a human mammary carcinoma, MAC117, showed a pattern of hybridization that differed both from that observed with DNA of normal human placenta and from that observed with the A431 squamous-cell carcinoma line, which contains amplified epidermal growth factor (EGF) receptor genes. In A431 DNA, four Eco RI fragments were detected that had increased signal intensities compared to those of corresponding fragments in placenta DNA (Fig. 1A). In contrast, MAC117 DNA contained a single 6-kilobase pair (kbp) fragment, which appeared to be amplified compared to corresponding fragments observed in both A431 and placenta DNA's (Fig. 1A). These findings indicate that the MAC117 tumor contained an amplified DNA sequence related to, but distinct from, the cellular erbB proto-oncogene.

By digestion of pMAC117 with Bgl I and Bam HI, it was possible to generate a single-copy probe homologous to v-erbB. This probe detected a 6-kb Eco RI fragment that was amplified in MAC117 DNA and apparently increased in A431 cellular DNA relative to normal DNA (Fig. 2B). The sizes of the fragment corresponded to the amplified 6-kb Eco RI fragment detected in MAC117 DNA by means of v-erbB (Fig. 2A). Hybridization to Southern blots containing serial dilutions of MAC117 genomic DNA indicated an approximate amplification of 5- to 10-fold when compared to human placenta DNA.

The nucleotide sequence of the portion of pMAC117 located between the Nco I and Acc I sites contained two regions of nucleotide sequence homologous to v-erbB separated by 122 nucleotides (Fig. 3). These regions shared 69 percent nucleotide sequence identity with both the v-erbB and the human EGF receptor gene. The predicted amino acid sequence of these regions was 85 percent homologous to two regions that are contiguous in the EGF receptor sequence. Furthermore, these two putative coding regions of the MAC117 sequence were each flanked by the AG and GT dinucleotides that border the exons of eukaryotic genes. These findings suggest that the sequence shown in Fig. 2 represents two exons, separated by an intron of a gene related to the erbB/EGF receptor gene.

The predicted amino acid sequence of the λMAC117 putative exons is homologous to the corresponding sequences of several members of the tyrosine kinase family. The most striking homology was observed with the human EGF receptor or erbB (Fig. 3). In addition, 42 percent to 52 percent homology with the predicted amino acid sequences of other tyrosine kinase-encoding genes was observed. At 25 percent of the positions there was identity among all the sequences analyzed (Fig. 3). A tyrosine residue in the λMAC117 putative coding sequence, conserved among the tyrosine kinases analyzed, is the site of autophosphorylation of the src protein (Smart et al., Proc. Natl. Acad. Sci. USA. 78:6013, 1981).

The availability of cloned probes of the MAC117 gene made it possible to investigate its expression in a variety of cell types. The MAC117 probe detected a single 5-kb transcript in A431 cells (Fig. 4). Under the stringent conditions of hybridization utilized, this probe did not detect any of the three RNA species recognized by EGF receptor complementary DNA. Thus, MAC117 represents a new functional gene within the tyrosine kinase family, closely related to, but distinct from the gene encoding the EGF receptor.

There is precedent for the identification of genes related to known oncogenes on the basis of their amplification in human tumors. For example, the high degree of amplification of N-*myc* in certain malignancies made it detectable by means of the *myc* gene as a molecular probe (Schwab, Nature 305:245, 1983; Kohl et al., Cell 35:349, 1983). In the present study, a five- to tenfold amplification of a v-*erbB*-related gene in the MAC117 mammary carcinoma made it possible to identify this sequence against a complex pattern of EFG receptor gene fragments.

The MAC117 coding sequence, as determined by nucleotide and predicted amino acid sequence, is most closely related to the *erbB*/EGF receptor among known members of the tyrosine kinase family. The two genes are distinct, however, as evidenced by the sequence diversity and transcript size. Detailed structural analysis of the complete coding sequence would further elucidate the role and function of this v-*erbB*-related gene.

To this purpose we have isolated cDNAs with a complexity of over 4.5 kb from the MAC117 mRNA (Kraus et al., EMBO Journal 6:605-610, 1987). A restriction map is shown in Fig. 5A. An oligo (dT) primed normal human fibroblast cDNA library (Okayama and Berg, 1983) was screened with a 0.8 kbp Acc I DNA fragment from the 3' terminus of a genomic clone of MAC117 (Fig. 1). The largest plasmid obtained, pMAC137, carried a 2-kbp insert comprising 1.5 kbp of 3' coding information and 3' untranslated sequence. The remaining coding information upstream was obtained from three phage clones, λMAC30, λMAC10' and λMAC14-1, identified in a randomly primed MCF-7 cDNA library (Walter et al., 1985; Fig. 5A).

Fig. 5B illustrates 3 probes, a, b, and c, representing the 5' end, a middle portion, and the entire coding region, respectively, which were employed in subsequent studies elucidating the role and function of this v-erbB-related gene.

To assess the role of MAC117 in human mammary neoplasia, we compared mRNAs of 16 mammary tumor cell lines to normal human fibroblasts, M413, and a human mammary epithelial cell line, HBL100. Increased expression of an apparently normal size 5-kb transcript was detected in 8 of 16 tumor cell lines when total cellular RNA was subjected to Northern blot analysis. An aberrantly sized erbB-2 mRNA was not detected in any of the cell lines analyzed (Kraus et al., EMBO Journal 6:605-610, 1987). To quantitate more precisely the amount of MAC117 transcript in eight mammary tumor cell lines which overexpress MAC117, serial 2-fold dilutions of total cellular RNA were subjected to dot blot analysis using human β actin as a control for the amount of RNA applied to the nitrocellulose filters. The highest levels of MAC117 mRNA, which ranged from 64- to 128-fold over that

of our controls, were observed in the cell lines MDA-MB453, SK-BR-3, MDA-MB361, and BT474. Moreover, MAC117 mRNA levels were increased 4- to 8-fold in four cell lines including BT483, MDA-MB175, ZR-75-30, and ZR-75-1 (Fig. 6).

To determine if the overexpression of MAC117 mRNA resulted in a steady state increase of its encoded gene product, we developed a specific immunoblot assay. Antisera were raised against a synthetic peptide whose sequence corresponded to a portion of the putative tyrosine kinase domain of MAC117. As this region is partially conserved between the encoded proteins of the EGFR and MAC117 genes, we tested its specificity using A431 and SK-BR-3 cell lines which overexpress EGFR or MAC117 mRNA, respectively. As shown in Fig. 7A, a specific band of ~ 185 kd was detected in extracts of SK-BR-3 but not in A431 cells. This band was not detected when the antibody was preincubated with the synthetic peptide corresponsing to its antigen. To estimate the relative amounts of MAC117 protein in different mammary tumor cell lines, immunoblot analysis was conducted using equivalent amounts of total cellular protein. As shown in Fig. 7B, an intense band of protein was detected in extracts of SK-BR-3 and a less intense but readily detectable band in extracts of ZR-75-1. No MAC117 protein was detected in extracts of MCF-7, a mammary tumor cell line, that did not display overexpression of erbB-2 mRNA. We interpret these results to indicate that substantially more erbB-2 protein is found in both SK-BR-3 and ZR-75-1 than in MCF-7 cells where the amount of protein escapes the sensitivity of the assay. These observations indicated that elevated mRNA levels of MAC117 are translated into MAC117 proteins. This demonstrated that gene amplification of MAC117 results in overexpression of mRNA and protein of MAC117 in human mammary tumor cells. Furthermore, increased mRNA and protein levels are observed in mammary tumor cells in the absence of gene amplification suggestive

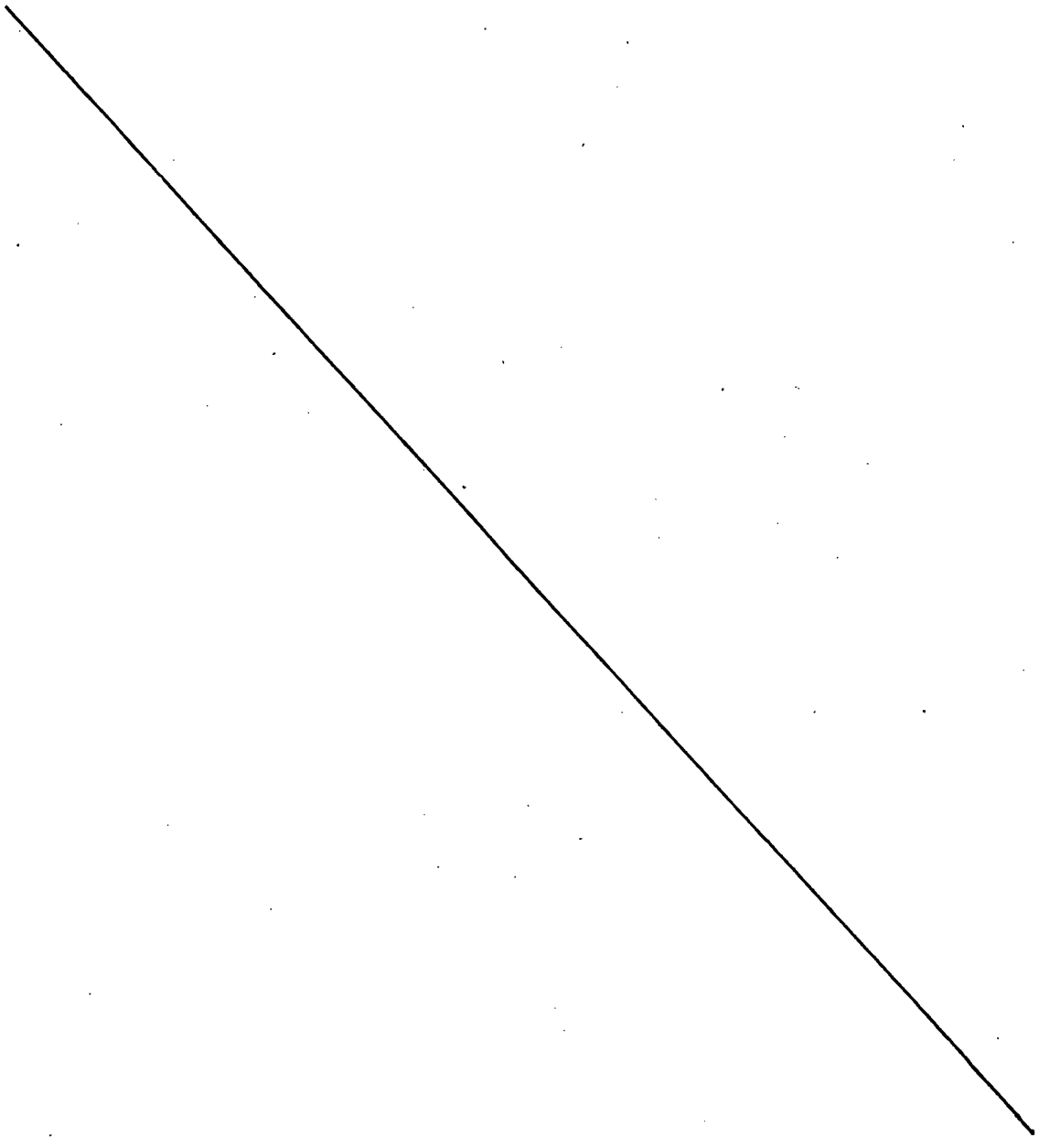
for an additional mechanism as a result of which mRNA and protein of our novel v-erbB-related gene can be found overexpressed in tumor cells (Kraus et al., 1987).

To directly assess the effects of MAC117 overexpression on cell growth properties, we assembled a full length normal human MAC117 clone from overlapping cDNA clones (~~Fig. 5~~ Figs. 5A,B) linked to the transcriptional initiation sequences of either the Moloney murine leukemia virus long terminal repeat (MuLV LTR) or the SV40 early promoter in expression vectors in order to express a normal coding sequence of MAC117 in NIH3T3 cells (Fig. 9) (DiFiore et al., Science 237:178-182 1987). Previous studies have indicated different strengths of LTR and the SV40 promoters in these cells (Gorman et al., Proc. Natl. Acad. Sci. USA 79, 6777, 1982). Because of the presence of the MuLV donor splice site close to the 5' LTR (Shinnick et al., Nature 293, 543, 1981), we engineered one of the LTR-based vectors (LTR-1/MAC117) to contain an acceptor splice site immediately upstream of the translation initiation codon of the MAC117 coding sequence (Fig. 9). This vector was constructed in order to ensure correct splicing of the message even if a cryptic splice acceptor site were present within the MAC117 open reading frame. In the SV40-based expression vector (SV40/MAC117) the erbB-2 coding sequence replaced the neomycin-resistance gene of pSV2/neo (Southern et al., J. Mol. Appl. Genet. 1, 327, 1982) (Fig. 9). To assess the biologic activity of our human MAC117 vectors, we transfected NIH/3T3 cells with serial dilutions of each DNA. As shown in Table 1, LTR-1/MAC117 DNAs induced transformed foci at high efficiency of 4.1×10^4 focus-forming units per picomole of DNA (ffu/pM). In striking contrast, the SV40/erbB-2 construct failed to induce any detectable morphological alteration of NIH/3T3 cells transfected under identical assay conditions (Table 1). Since the SV40/erbB-2

construct lacked transforming activity, these results demonstrated that the higher levels of MAC117 expression under LTR influence correlated with its ability to exert transforming activity. To compare the growth properties of NIH/3T3 cells transfected by these genes, we analyzed the transfectants for anchorage-independent growth in culture, a property of many transformed cells. The colony-forming efficiency of a LTR-1/MAC117 transformant was very high and comparable to that of cells transformed by LTR-driven v-H-ras and v-erbB (Table 1). Moreover, the LTR-1/MAC117 transfectants were as malignant in vivo as cells transformed by the highly potent v-H-ras oncogene and 50-fold more tumorigenic than cells transfected with v-erbB. In contrast, SV40/MAC117 transfectants lacked anchorage-independent growth in vitro and did not grow as tumors in nude mice even when 10^6 cells were injected (Table 1).

To compare the level of overexpression of the 185-kd protein encoded by MAC117 in human mammary tumor cell lines possessing amplified MAC117 genes with that of NIH/3T3 cells experimentally transformed by the MAC117 coding sequence, we compared MAC117 specific protein amounts by Western blotting (DiFiore et al., Science 237: 178-182 1987). An anti-MAC117 peptide serum detected several discrete protein species ranging in size from 150 to 185 kd in extracts of MDA-MB361 and SK-BR-3 mammary tumor cell lines, as well as LTR/MAC117 NIH/3T3 transformants (Fig. 10). The relative levels of the 185-kd MAC117 product were similar in each of the cell lines and markedly elevated over that expressed by MCF-7 cells, where the 185-kd protein was not detectable under these assay conditions (Fig. 10). Thus, human mammary tumor cells which overexpressed the MAC117 gene demonstrated levels of the MAC117 gene product capable of inducing malignant transformation in a model system.

Overexpression of proto-oncogenes can cause cell transformation in culture and may function in the development of human tumors. Amplification of a normal



ras gene or its increased expression under the control of a retroviral long terminal repeat (LTR) induces transformation of NIH 3T3 cells (Chang et al., Nature 297:479, 1982). Expression of the normal human sis/PDGF-2 coding sequence in NIH 3T3 cells, which do not normally express their endogenous sis proto-oncogene, also leads to transformation (Gazit et al., Cell 39:89, 1984; Clarke et al., Nature 308:464, 1984). In Burkitt lymphoma, a chromosomal translocation involving myc places its normal coding sequence under the control of an immunoglobulin gene regulatory sequence. The resulting alteration in myc expression is likely to be causally related to tumor development (Nishikura et al., Science 224:399, 1984). The observation of amplification of myc or N-myc in more malignant phenotypes of certain tumors has supported the idea that overexpression of these genes can contribute to the progression of such tumors. The erbB/EGF receptor gene is amplified or overexpressed in certain tumors or tumor cell lines. The five- to tenfold amplification of the v-erbB-related gene of the present invention in a mammary carcinoma indicates that increased expression of this gene may have provided a selective advantage to this tumor. The isolation of a new member of the tyrosine kinase gene family amplified in a human mammary carcinoma in accordance with the present invention, makes possible the elucidation of the role of this gene in human malignancy.

Use of Specific Nucleic Acid Probes

As demonstrated in Figures 1 and 2, the isolation and use of a Bgl I to Bam HI restriction fragment of pMAC117 to specifically detect the gene and its mRNA product has been set forth. The importance of this technique, involving this probe and others like it, is that the biological functions of the gene described here can be determined and these functions related to practical application, some of which are listed below.

1. Isolation of cloned cDNA. This involves the use of probes specific for the gene described herein; an example is the Bgl I-Bam HI fragment of pMAC117. These probes are made radioactive by standard techniques, such as those noted above, and screening of the libraries of cDNA clones is done using standard methods analogous to those described in "Cloning of λMAC117" above. This approach was employed to clone cDNA comprising the entire coding region of this gene, the restriction map of which is shown in Fig. 5A.

2. Use of cDNA clones. Due to the fact that cDNA clones contain complete information for encoding the protein, these cDNA clones provide a "second generation" of specific probes for the gene described herein. Such probes are shown in Fig. 5B. Their application for hybridization analysis is demonstrated in Fig. 6 and Fig. 8. As shown in Fig. 8, the availability of probes, such as probe 2 in Fig. 5B, facilitates the comprehensive hybridization analysis of the entire coding

region of this gene or any defined part of it. In addition, the complete coding information allows the expression of the protein product in a heterologous system. Such systems utilize strong and/or regulated transcription promoters placed in such a way as to direct overexpression of the gene. Techniques for accomplishing expression of the gene are well known in the art and can be found in such publications as Rosenberg et al., Methods in Enzym. 101, 123 (1983); Guarante, L., Methods in Enzym. 101, 181 (1983). The coding region of our novel v-erbB-related gene was overexpressed under the transcriptional control of MuLV-LTR or SV40 early promoter. Thereby, high expression levels were achieved with MuLV-LTR which caused the neoplastic transformation of transfected cells. These cells can be used as a source to rescue infectious recombinant virus which might prove useful to infect heterologous cells not susceptible to DNA transfection. In addition, these cells serve as a source for high and defined levels of antigen for this novel v-erbB-related gene.

3. Preparation of antibodies specific for the protein product of the gene. Of course, the identification and knowledge of the gene allows its product, protein, for example, to be detected. Poly- or monoclonal antibodies are prepared against said

protein by standard techniques, often by commercially available services. The critical reagent in the production of antibodies is the antigen (protein) used. In this case, the antigens are either the peptides chemically synthesized by standard and commercially available techniques according to the predicted amino acid sequences derived from the nucleic acid sequence of the gene or its corresponding cDNA. Another potential antigen is the protein itself encoded by the gene and purified from the heterologous expression systems as described above. The antibodies are then employed by standard immunological techniques for the specific detection or diagnostic purposes. Such antibodies were raised against a peptide representing amino acids 35 through 49 of the peptide ~~sequence of claim 4~~. The specificity of these antibodies in detecting the gene product of this ~~novel v-erbB-related gene in~~ demonstrated in Fig. 7A. As shown in Fig. 7B and Fig. 10, these antibodies can be utilized to detect the over-expression of the protein product of our novel v-erb-B-related gene in human mammary tumor cells.

Further Applications of the Gene:

Having the knowledge of the gene allows preparing specific nucleic acid probes to detect the gene described here or its mRNA product. The probes are, of course,

derived from the gene, such as the Bgl I-Bam HI fragment of pMAC117 used in Figures 1 and 4, or alternatively such probes are derived from other regions of the gene or its corresponding cDNA corresponding cDNA, as shown in Fig. 5A. The use of nucleic acid probes in the molecular diagnosis of human cancer has been documented (Taub et al., Proc. Natl. Acad. Sci. USA 79, 7837 (1983); Schwab et al., Proc. Natl. Acad. Sci. USA 81, 4940 (1984)). The finding that the gene described here is amplified in a human mammary carcinoma indicates that alterations occur to this gene in human disease. Thus, detection of the amplification or increase expression of this gene provides useful diagnostic tools for the detection and treatment of human mammary carcinoma or other malignancies resulting from the v-erbB related gene. Hence, diagnostic kits which contain as their principal component specific nucleic acid probes for this gene or its mRNA transcript are of commercial value. The probe is used in analyses similar in concept to those shown in Figure 1 and Figure 4 for the detection of gene amplification structure or the expression of mRNA.

Specific antibody reagents (as described above) capable of detecting the protein product of the gene described herein are employed in a way similar to the use of specific nucleic acid probes. In other words, the expression of aberrant forms and amounts of a gene product is a measure of the related neoplastic condition

(Nishikura et al., Science 224, 399 (1984); Srivastava, et al., Proc. Natl. Acad. Sci. USA 82, ,38-42 (1985)). The detection of the aberrant expression of the protein product of the gene is of importance in the diagnosis of human cancers. As shown in Fig. 7 and Fig. 10, antibodies generated against peptides derived from ~~form~~ parts of the amino acid ~~sequence of claim 4~~ specifically detect the protein product of the gene ~~described in claim 1~~ in human tumor cells. Antibody reagent (produced as described above) is, of course, the critical reagent of the diagnostic kits for this purpose. Such antibody reagents are then employed in such standard methodologies as immunoprecipitation, western blot analysis, immunofluorescence analysis and the like well known in the art. The determination of amplification in a human mammary carcinoma of the gene described here indicates that overexpression (or other abnormality) of the protein product of this gene is functionally important, thus diagnostically relevant. This relevance is further substantiated by the observations that gene amplification of this gene is associated with overexpression of its mRNA and protein in human mammary tumor cells and that protein levels observed in human mammary tumor cell lines exhibiting gene amplification of this gene are sufficient to induce neoplastic transformation of NIH/3T3 cells in vitro. Furthermore, a recent report (Slamon et al., Science 235:177-181, 1987) correlates gene amplification of this novel erbB-related gene with a reduced disease free survival in breast cancer patients, suggesting the potential usefulness of analysis of this gene for its gene product as a diagnostic parameter in the clinical setting

A diagnostic test in accordance with the present invention involves, for example, material obtained by surgical biopsy of potential tumor material. Such material is then analyzed by one or more procedures as follows.

1. DNA is isolated from the sample by standard methods (see above). The DNA is then analyzed by established methods, such as Southern blot hybridization using standard techniques similar to those used in the analysis shown in Figure 2. Gene-specific probes (described above) are made radioactive by standard techniques and used for detecting genetic abnormalities. Such abnormalities include gene amplification, as seen in the MAC117 tumor sample and tumor cell lines in Fig. 8. or gene rearrangement, as detected by aberrantly migrating bands of hybridization.

2. RNA is isolated from the tumor sample by standard methods (see above). This RNA is analyzed by blot hybridization techniques similar to those described in Figure 4. Gene-specific probes (described above) are made radioactive by standard techniques and used for detecting the mRNA products of the erbB-related gene described here. Such abnormalities include overexpression or abnormal forms of RNA. Overexpression of an apparently normal sized mRNA is shown in 8 human mammary tumor cell lines in Fig. 6. In addition, mRNA amount may also be quantitated by spot hybridization

procedures in which serial dilutions of RNA ~~is~~ are fixed to nitrocellulose filter and the mRNA of v-erb-B-related gene described here detected by hybridization. Such a procedure has been employed in Fig. 6B. The foregoing techniques are standard. This allows detection of mRNA overexpression or alteration of structure.

When antigens or proteins (polypeptides) are to be analyzed, the proteins are separated according to molecular size, for example by gel electrophoresis, transferred to nitrocellulose membranes and the protein product of the erbB-related gene described here detected by reaction with specific antibodies, described above. Such a test is able to detect alterations in the quantity of protein as well as abnormal protein forms. With such an approach protein levels of the v-erb-B-related gene have been detected in human mammary tumor cell lines (Fig. 7, Fig. ~~11~~ 10).

In addition, specific antibodies may be used in the analysis of histological sections. These techniques, which are well known for other antibody specificities, involve the thin sectioning of biopsied material from a potential tumor, followed by reaction with specific antibodies. The antibody-antigen reaction is then made visible by a variety of standard methods including labeling with fluorescently tagged or ferritin tagged

second antisera and the like. Such detection systems allow the detection of the localized aberrant display of the protein product of the erbB-related gene described here.

In addition, although the demonstated genetic abnormality (shown in Figure 1) of the gene described here occurs in human mammary carcinoma, genetic abnormalities may also be associated with other clinically important syndromes of neoplastic or other origin. Genetic abnormalities have long been known to be involved in thalassemias, for example.

Knowledge of the erbB-related gene described here also makes possible a means of cancer treatment. If it is found that some cancers display abnormally high quantities of the gene product on their surface, such tumors can be treated with antibodies specific for the gene product which ~~has~~ have been conjugated to a toxic substance, such as radioactive markers, biological modifiers or toxins and the like. Another treatment modality involves a similar assumption of overexpression. In this approach, a specific natural product, even if unidentified but which has high binding affinity for the protein product of the gene described

here is used to target toxins to the tumor cells. This treatment modality is supported by the finding, reported here, of distinct but limited homology of this gene product to the EGF receptor. If a ligand analogous to EGF exists for the erbB-related gene described here, it may serve as such a targeting agent.

Diagnostic kits for the detection of the protein product of the erbB-related gene. Kits useful for the diagnosis of human cancers having abnormalities of this gene are now disclosed.

a) Kits designed to detect the protein by immunoblotting These kits preferably comprise containers containing (a) homogenization solution (50 mM Tris-HCl pH 7.5, 1% sodium dodecyl sulfate and 0.1% β-mercaptoethanol) for the extraction of protein sample from biopsied material from putative tumors; (b) reagents for the preparation of immunoblots of the protein samples (acrylamide gels are prepoured and contain 7.5% acrylamide, .025% bis acrylamide, 0.38 M Tris-HCl pH 8.8, 0.1% sodium dodecyl sulfate; the nitrocellulose sheets will be formed to the gel size; and transfer buffer 0.25 M Tris-glycine pH 8.8, 30% methanol); specific antibody reagents for the detection of the

protein product of the erbB-related gene (antisera directed against the protein product of erbB- related gene described here and reaction buffer containing 0.1 M Tris-HCl pH 7.5, 5.0 M EDTA, 0.25% gelatin, 0.1% nonidet P-40); and reagents and instructions for the visualization and interpretation of antibody-antigen interaction (these include radioactive protein A; biotin conjugated second antiserum, or peroxidase conjugated second antiserum). While this kit includes components ordinarily found and well known in the art, the critical component is the gene product-specific antibodies and buffers or media for performing immunological tests. The antibodies are derived or prepared as described above from either the peptide sequence predicted from the nucleotide sequence of the gene or its mRNA or from the protein product itself through standard immunization procedures.

b) Kits designed for the detection of the protein product of the erbB-related gene in tissue sections. Such kits include instructions for preparation of sections; instructions and standard reagents for the preparation of slides for microscopy; H_2O_2 for removal of endogenous peroxidase; instructions for incubation with antibodies specific for the protein

product of the erbB-related gene described here in a buffer solution preferably containing phosphate buffered saline; and second antibodies for detection (these may be coupled to peroxidase, biotin, or ferritin); and instructions for visualization of detection complex. In addition the kits may include: reagents and instructions for the preparation of sections from biopsied putative tumor material; specific antibody reagents for the protein product of erbB-related gene described here and instructions for its reaction with the tissue section; and reagents and instructions for the detection of the protein-antibody interaction either by immunofluorescence, ferritin conjugated second antibodies or other standard methods well known in the art.

A Method for the Treatment of Human Cancers which Express High Levels of the Protein Product of the Gene Described herein.

This method involves administering to the patient one of two types of reagent which preferentially binds cells expressing high levels of the protein product of the erbB-related gene described here. These reagents are either antibodies directed against the protein product or

a ligand, which is likely to exist because of the homology of the gene to a growth factor receptor. The ligand is isolated by standard techniques using the intrinsic protein kinase activity of the protein product of the erbB-related gene. Extracts of body fluids and cell culture supernatants are incubated with the protein and γ-^{32}P ATP. The presence of ligand is inferred by incorporation of ^{32}P into the protein. The ligand is then purified by standard techniques such as ion exchange chromatography, gel permeation chromatography, isoelectric focusing, gel electrophoresis and the like. The natural ligand or antibody is tagged with one or more agents which will cause injury to cells to which they bind. Such tagging systems include incorporation of radioactive or biological toxins. The present discovery of amplification of the erbB-related gene makes it likely that some tumors carry large amounts of the corresponding protein. Hence, the two type-specific agents will bind in larger amounts to the protein present in the body and thus direct the toxic effects of the reagents to these cells.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.